

CMBL – CRJ/JJR
6/10/08

Protocol: Hygromycin B Selection (FAK null cells)

Materials:

- Hygromycin B (Invitrogen cat# 10687-010) 50 mg/ml
- Complete medium: α MEM w/out nucleosides (Invitrogen cat# 12561) + 15 % Fetal Bovine Serum + 1% Pen/Strep
- Syringe (sterile) / 0.22 μ m syringe filter (sterile)
- Sterile centrifuge tubes
- Tube rack, gloves, 70 % ethanol, etc. – other general items used while working in the sterile hood

Procedure:

1. Sterile filter and aliquot Hygromycin B stock solution
2. Prepare medium containing Hygromycin B prior to each use (personal preference)
 - add 350 μ l of Hygromycin B (50 mg/ml) to 49.65 mL of complete media (final concentration of 350 μ g/ml – FAK -/- cell specific)
3. Mix by pipetting up and down before adding to cells.
4. Change media on cells under hygromycin B selection every 3 days (hygromycin B degrades @ 37°C incubator)

Note: Hygromycin B and α MEM without nucleosides may be purchased from BioStores on the Stanford campus